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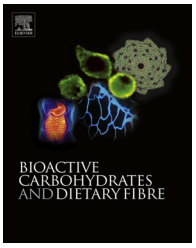

Surface properties of semi-synthetic enteric coating films: Opportunities to develop bio-based enteric coating films for colon-targeted delivery

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ABSTRACT

This study investigated the surface properties of the semi-synthetic enteric coatings materials for colon-targeted bioactive delivery. The enteric coating materials were prepared by combining nanoscale resistant starch, pectin, and carboxymethylcellulose. The surface properties of the coating materials were characterized by atomic force microscopy for barrier properties, physical stability, and the viscoelastic properties: the surface of the coatings was characterized in terms of root-mean square roughness (RMS), peak- to-valley height (R_z), surface skewness (R_{sk}), and surface kurtosis (R_{ku}). The coating with pure nanoscale resistant starch was used as a control, which showed poor surface properties compared to the other films. However, the enteric coating films with nanoscale resistant starch: pectin 90:10 and nanoscale resistant starch: carboxymethylcellulose 10:90, showed very good barrier properties, visoelasticity, and physical stability. Therefore, the results of study suggest that the nanoscale resistant starch, pectin, and carboxymetylcellulose could be used to produce novel enteric coatings with good surface properties towards targeted delivery of bioactive compounds to the colon.

1. Introduction

Enteric coating materials for targeted bioactive compounds delivery should be effective, efficient, and safe (Parveen, Misra, & Sahoo, 2012). The enteric coatings should also be stable during their passage through the gastro intestinal (GI) tract. In addition, the enteric coatings that are biocompatible will have greater appeal in human applications (Parveen et al., 2012; Dimantov, Greenberg, Kesselman, & Shimoni, 2004). Thus, the design and selection coating materials is critical for targeted deliveries of pharmaceutical and nutrients.

Development of coatings for colon-targeted delivery has progressed rapidly in the recent years; use of nanoparticles has become popular in the development of enteric coatings for targeted delivery. Most of the coating materials that are currently used, are derived from natural, semi-synthetic or synthetic sources (Nazzaro, Orlando, Fratianni, & Coppola, 2012; Dimantov et al., 2004). However, lately, a combination

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of resistant starch (RS), a fraction of starch that resists the digestion in the small intestine (Gibson & Roberfroid, 1995), with pectin and cellulose (ethylcellulose and carboxynethylcellulose) has shown potential value towards colon-targeted delivery (Dimantov et al., 2004; Liu, Fishman, Kost, & Hicks, 2003; Macleod, Fell, & Collett, 1997). The efficiency of these coatings can be further increased by reducing the particle size of one or more compounds, which are combined to form enteric coatings; the reduced particle sizes of the components can retain the bioactive compounds until they reach the colon (Dimantov, Kesselman, & Shimoni, 2004; Sivapragasam, Thavarajah, Ohm, & Thavarajah, 2014 and Sivapragasam, Thavarajah, Ohm, Khaitsa, & Thavarajah, 2014). Therefore, a potential approach to design efficient coating materials for colon- targeted delivery.

The overall goal of this study was to develop coatings with pectin and carboxymethylcellulose with nanoscale RS to determine their barrier properties, viscoelasticity, and diffusivity properties; semi-synthetic enteric coatings were produced with different combinations of RS nanoparticles, pectin, and carboxymethyl cellulose. The RS was isolated from soybean meal (SBM) (Sivapragasam, Thavarajah, Ohm, Khaitsa, & Thavarajah, 2014), soybean meal is a by-product of soybean oil processing, and used to produce nanoscale RS. Surface properties of the coatings were characterized by atomic force microscopy (AFM).

2. Materials and methods

2.1. Materials

Soybean meal was obtained from Northern Crops Institute (NCI), Fargo, North Dakota. Pectin, carboxymethylcellulose, and enzymes were purchased from Sigma Aldrich Co (St. Louis, MO). Glass slabs (3″ × 6″) were purchased from a local glass ware supplier at Fargo, North Dakota.

2.2. Coating materials

Resistant starch from soybean meal was isolated from a previously reported procedure (Sivapragasam et al., 2014b). Soybean meal was defatted using Soxhlet extraction. Defatted sample were mixed with 150.0 ml extraction buffer (50 mM ethylenediaminetetraacetic acid (EDTA), 50 mM sodium acetate, and 50 mM sodium oxalate at pH 5.2). The mixture was stirred for 60 minutes at 70 °C using a magnetic stirrer plate (VWR International LLC, West Chester, PA). This was followed by centrifugation for 15 minutes at 5000 rpm in a Beckman J2-HS (Beckman Coulter Inc., Brea, CA).The supernatant was mixed with ethanol to a final alcohol concentration of 70%. The sample was centrifuged again under the same conditions and the resulting precipitate was collected and dissolved in 50 mM sodium hydroxide with heating to 70 °C. Non-soluble particles were removed by filtration through Whatman filter paper number 4 (Whatman International Ltd., Maidstone, UK) and pectin was precipitated from clear solution by addition of solid barium chloride. The sample was then centrifuged for 10 min at 6000 rpm and the supernatant was mixed with ethanol to a final alcohol concentration of 70%. The sample was again centrifuged, with the precipitate air dried. The resulted sample was subjected to enzymatic assay to isolate resistant starch.

Table 2.1 – Composition of the enteric coatings.

Resistant starch (%)	Pectin (%)	Resistant starch (%)	Carboxymethylcellulose (%)
100	0	100	0
90	10	90	10
80	20	80	20
70	30	70	30
60	40	60	40
50	50	50	50
40	60	40	60
30	70	30	70
20	80	20	80
10	90	10	90
0	100	0	100

The soybean meal resistant starch was used to prepare nanoparticles by mechanical agitations. The resistant starch solution was prepared with ethanol at 1:5 (w/v). The solution was subjected to mechanical agitation by sonicating (40 kHz) using an ultra sonicator (Branson Inc., Chicago, IL) at 40 °C for 5 h.

The resistant starch nanoparticles were dissolved in 50 mM NaOH (3% w/v); the pectin and carboxymethylcellulose were individually dissolved in Millipore water (3% w/v). Different combinations of the enteric coatings were prepared as shown in Table 2.1.

2.3. Surface characterization

The thickness of the coatings casted on the glass slabs were 75 μm. The surface characterization was studied by AFM (Veeco technologies 3100, Santa Clara, CA) in a tapping mode. Scans were performed in air. The cantilever resonance frequency was 47–76 kHz with a force constant of 12.64 N m^{-1}. Sampling resolution were 512 × 512 points. Three different representative spots were selected and the measurements were averaged across the representative spots. The root mean square roughness (RMS) was directly obtained from the image. The peak-to-valley height (R_z), surface skewness (R_{sk}), and surface kurtosis (R_{ku}) were calculated using the following formulas (Stawikowska & Livingston, 2013):

$$Rz = Z\max - Z\min$$

$$R_{sk} = \frac{1}{nRq3}\sum_{i=1}^{n} Zi3$$

$$R_{ku} = \frac{1}{nRq4}\sum_{i=1}^{n} Zi4$$

3. Results and discussion

3.1. Surface roughness analysis

Surface roughness can be studied by root- mean square roughness (R_q) and peak-to-valley height (R_z). Figs. 3.1 and 3.2 show the changes in the R_q and R_z with different combinations of pectin and carboxymethylcellusose with resistant starch (RS); both R_q and R_z showed similar patterns. As shown in Fig. 3.1,

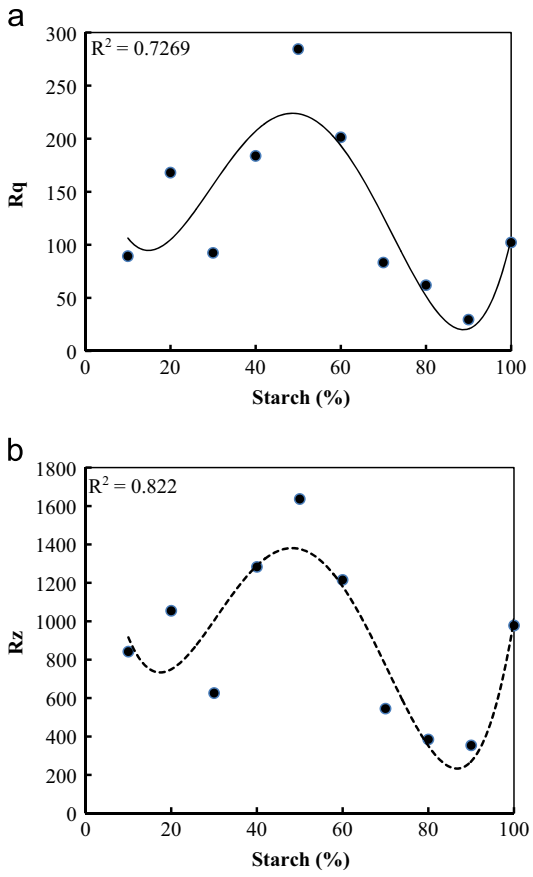

Fig. 3.1 – Changes in (a) R_q and (b) R_z, with different combinations of RS and pectin.

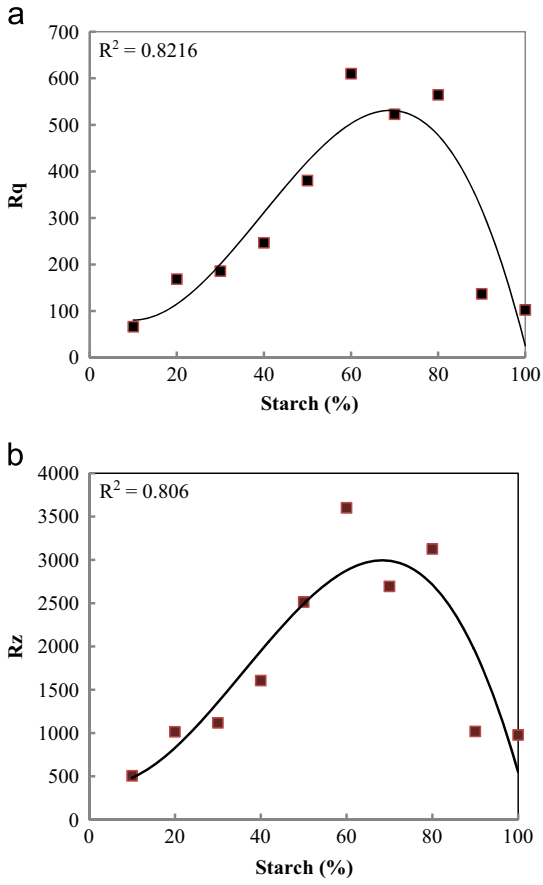

Fig. 3.2 – Changes in (a) R_q and (b) R_z, with different combinations of RS and carboxymethylcellulose.

combination of RS and pectin showed an initial decrease in the R_q and R_z with decreasing amount of RS; R_q is lower with the addition of 90–70% of RS compared to the control (100% RS). However, the R_q was higher when the amount of RS was 60–40% and 20%. Previous studies (Hilal, Al-Zoubi, Darwish, & Mohammad, 2005; Johnson, Al Malek, Al-Rashdi, & Hilal, 2012; Kuhlmeier, Rodda, Kolarik, Furlong, & Bilitewski, 2003; Garcia-Ayuso, Vázquez, & Martínez-Duart, 1996) have suggested better barrier properties for moisture and gas, for the films with lower R_q and R_z. Although, uniformity in the R_q and R_z is not seen with different combinations of RS and pectin – the lower values for both R_q and R_z – compared to control – could be related to barrier properties. Thus, the lower R_q and R_z values for different combinations of pectin and RS films suggest better barrier properties.

The R_q and R_z with different combinations of RS and carboxymethylcellulose are shown in Fig. 3.2. A uniformity in both R_q and R_z is seen with increasing amounts of carboxymethylcellulose. Addition of carboxymethylcellulose up to 40% showed an increase in both R_q and R_z. The lowest R_q and R_z values were seen only with the film having a combination of 90:10 carboxymethylcellulose: RS; this is the only films with a combination of RS and carboxymethylcellulose, which shows better barrier properties.

RS is made up of short oligosaccharides. The physical stability of these short chains can be increased by different combinations of suitable polymers (Dimantov et al., 2004). Addition of pectin and carboxymethylcellulose to the short oligosaccharides could decrease the mobility of the molecules to increase the physical stability (Dimantov et al., 2004; Wakerly, Fell, Attwood, & Parkins, 1997; Dimantov et al., 2004). The induced chain entanglements of these polymers can trigger the chains to coil up and decrease the surface area, which in turn results in lower R_q and R_z – to posses' better moisture and gaseous barrier properties (Stawikowska & Livingston, 2013). The induced chain entanglement of the nanoparticles with pectin and carboxymethylcellulose aids the integrity of the surface (Fig. 3.3); this polymer chain entanglement is important for the stability of the coatings. This study clearly shows that, in contrast to pectin, higher amounts of carboxymethylcellulose (90:10 carboxymethylcellulose: RS) is needed to enhance the physical stability of the films; however, the non-uniformity behavior with pectin and RS may need explanation by using molecular simulation models.

3.2. *Surface skewness analysis*

The surface skewness (R_{sk}) was negative for the control film (100% RS). Positive skew values were seen for the films with pectin: RS 10:90 and carboxymethylcellulose: RS 90:10 (Fig. 3.3). All the other films showed negative skew values. Films with positive skewness, where peaks are dominant are an indication of the non-porous surface of the film (Fig. 3.4b and c). In contrast, the negative skewness values, where

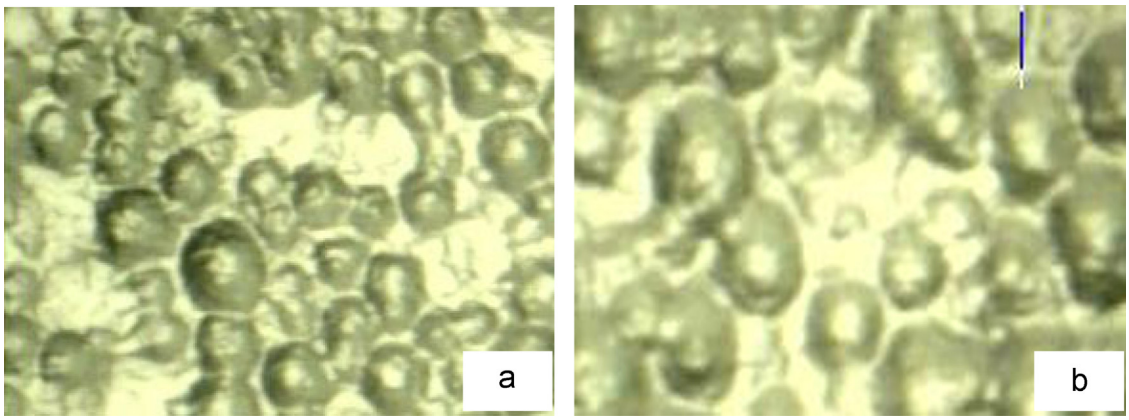

Fig. 3.3 – (a) Surface appearance of (a) pectin: RS 10:90 and (b) carboxymethylcellulose: RS 90:10.

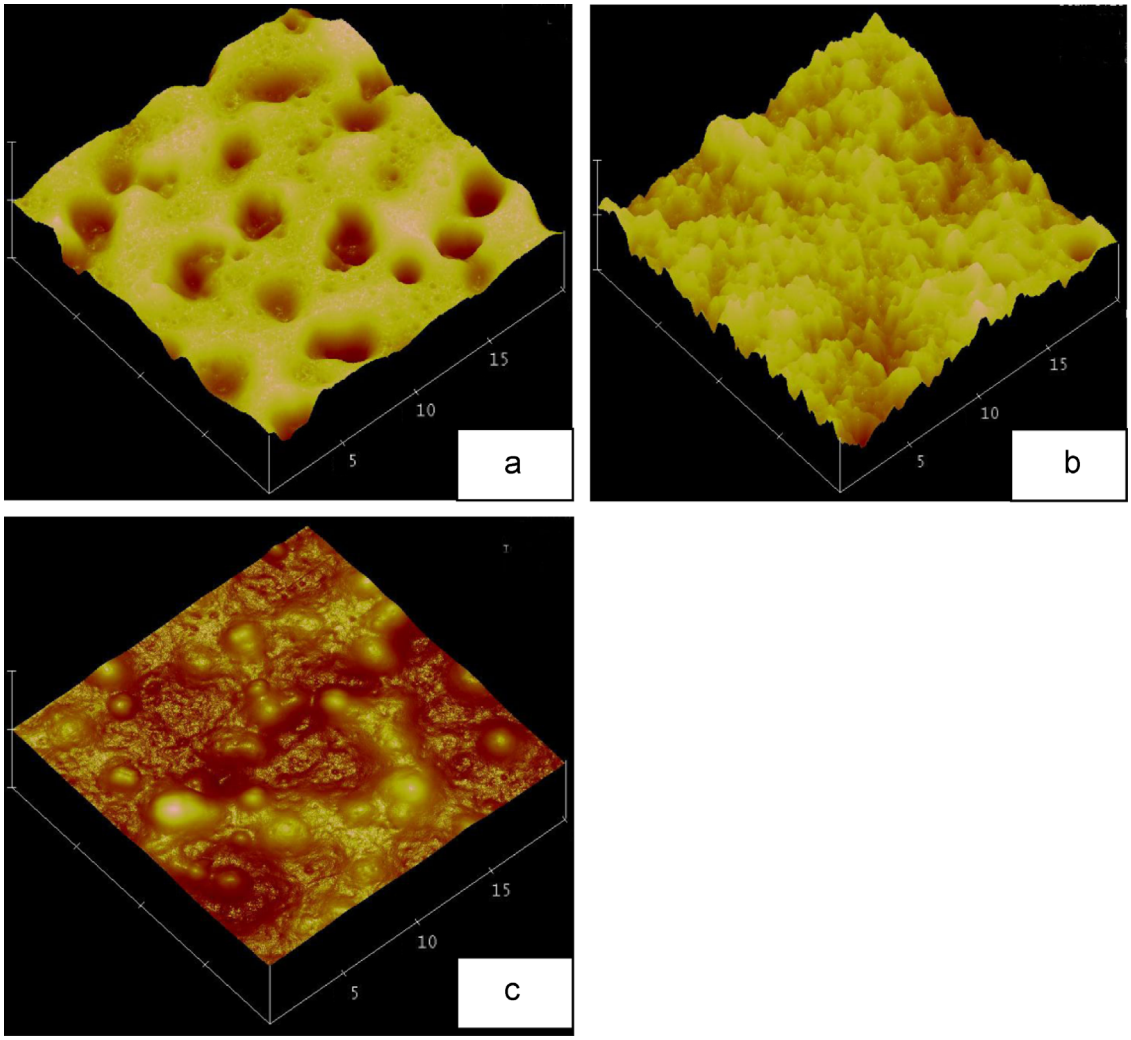

Fig. 3.4 – (a) negative skew for the control (100% resistant starch nanoparticle), (b) positive skew for resistant starch nanoparticle: pectin 90:10, and (c) positive skew for resistant starch nanoparticle: carboxymethylcellulose 10:90.

valleys are dominant, indicate a porous surface (Fig. 3.3a). Previous studies (Hilal et al., 2005; Stawikowsa & Livingston, 2013) have reported that the surfaces with high porosity show higher diffusivity, which in turn could lead to a high permeability of moisture and gas— resulting in poor barrier properties. However, surfaces that is less porous are densely packed and less compliant, which leads to a stiffer surface with better barrier and viscoelastic property (Hilal et al., 2005; Stawikowsa & Livingston, 2013). Although the skewness values for better barrier properties are comparable with R_q and R_z – comparison of R_q, R_z, and R_{sk} suggest that the films with pectin: RS 10:90 and carboxymethylcellulose: RS 90:10

would be better films, compared to other films, with enhanced physical stability, better barrier properties, and viscoelasticity.

3.3. *Surface kurtosis analysis*

Surface kurtosis (R_{ku}) for different combinations of pectin and carboxymethylcellulose with RS were studied. Surface kurtosis is an indication of the sharpness of the height distribution of the surface of the film (Stawikowsa & Livingston, 2013), which is not extensively used to characterize the surface of the films. However, this study shows (data not shown) that only few films with different combinations of pectin and RS had sharp height distribution (Rku > 3) – while films with a combination of carboxymethylcellulose and RS showed flat repetitive surface (Rku < 3).

4. Conclusion

The surface characterization of the films clearly showed that the RS needs additional physical support to possess better barrier and viscoelastic properties. The R_q, R_z, R_{sk}, and R_{ku} suggest that the films with pectin: RS 10:90 and carboxymethylcellulose: RS 90:10 have better barrier properties for moisture and gas with stiffer surface (lower viscoelascticity). The enhanced physical stability of the films could be used for the packaging of bioactive compounds targeted for colon – due to the unique combinations of the films – that is required for the delivery to the colon. However, at this point the potential use of these materials for colon-targeted delivery is only a speculation. Further studies, such as dissolution and digestion studies, are required to confirm the chemical stability of the films during the transit through the gastrointestinal tract towards efficient colon targeted delivery.

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